Management of Xanthomonas Leaf Blight of Onion with a Plant Activator, Biological Control Agents, and Copper Bactericides

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ABSTRACT

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Xanthomonas leaf blight (*Xanthomonas axonopodis* pv. *allii*) is a yield-limiting disease of onion (*Allium cepa*) in the western United States. Frequent applications of copper-based bactericides amended with an ethylenebisdithiocarbamate fungicide (e.g., maneb or mancozeb, class B2 carcinogens) provide some disease suppression, but strategies to reduce conventional bactericide use are needed to minimize grower costs, environmental impact, and public exposure to class B2 pesticides. Applications of acibenzolar-*S*-methyl reduced in planta and epiphytic populations of *X. axonopodis* pv. *allii* as effectively as applications of copper hydroxide-mancozeb in growth chamber studies. Under field conditions, four weekly applications of acibenzolar-*S*-methyl reduced severity of Xanthomonas leaf blight as or more effectively than 9 to 12 weekly applications of copper hydroxide or copper hydroxide-mancozeb. Acibenzolar-*S*-methyl applications did not increase bulb yield or grade compared with copper bactericide treatments. However, bulb yield was reduced 22 to 27% when 10 weekly applications of acibenzolar-*S*-methyl were made in the absence of disease. Application of a commercial formulation of both *Pantoea agglomerans* strain C9-1 and *Pseudomonas fluorescens* strain A506 reduced severity of Xanthomonas leaf blight in field experiments. Weekly copper hydroxide applications starting 1 to 2 weeks before bulb initiation were as effective as weekly applications started 3 to 4 weeks before bulb initiation, irrespective of the maneb rate used. Integration of acibenzolar-*S*-methyl and biological control agents with copper hydroxide in a carefully timed spray program may eliminate the use of the class B2 carcinogens maneb and mancozeb on onion without compromising efficacy for management of Xanthomonas leaf blight.

Additional keywords: bacterial leaf blight, *Erwinia herbicola*, integrated pest management, onion bacterial blight, *Xanthomonas campestris* pv. *allii*

Xanthomonas leaf blight of onion (*Allium cepa*), caused by the bacterium *Xanthomonas axonopodis* pv. *allii*, is a yield-limiting disease of onion in Colorado (32,33). Disease symptoms can be varied, but generally appear as lenticular-shaped chlorotic spots that quickly develop into water-soaked lesions during rainy or humid conditions. Symptoms progress to chlorotic streaks, necrosis, and tip dieback, resulting in stunting of plants and reduction or cessation of bulb development. Lesions are most prominent on the flattened sides of leaves. A bulb rot has not been reported in association with this pathogen, but yield losses can be significant in Colorado (33).

Few management strategies have been developed for Xanthomonas leaf blight. Crop rotation to nonhosts (5,13,21,27), use of resistant cultivars (21), and planting pathogen-free seed have been suggested (28,29). Commercial cultivars resistant to Xanthomonas leaf blight have not been identified for local producers in Colorado (H. Schwartz, *unpublished data*), where the disease continues to cause losses in some years.

Copper bactericides amended with an ethylenebisdithiocarbamate (EBDC) fungicide can suppress Xanthomonas leaf blight and other bacterial diseases of onion in Colorado, but applications must be applied preventatively and regularly to be effective (33). Consequently, growers may make eight or more copper/EBDC applications per season to suppress disease. This approach to disease management is expensive for onion growers and depends heavily upon EBDC fungicides. These fungicides are considered class B2 carcinogens and may have their registrations cancelled in the future (12,38). Additionally, this approach to disease management is not likely to be sustainable because it relies heavily upon chemical inputs prone to resistance development. Copper resistance has not been observed among strains of *X. axonopodis* pv. *allii* prevalent in Colorado (8), but it has been reported in Barbados (22). Bactericide resistance is widespread among phytopathogenic bacteria (4,19,35), and copper resistance is likely to appear in populations of *X. axonopodis* pv. *allii* if onion growers continue to rely upon copper bactericides for disease suppression. Copper bactericides alone provide little control of copper-tolerant bacteria (18,31). Therefore, new management strategies for Xanthomonas leaf blight are needed to delay or prevent the development of copper tolerance in populations of *X. axonopodis* pv. *allii*, and to minimize EBDC fungicide use and onion production costs.

Copper bactericide and fungicide use could be reduced in onion crops in Colorado by improving the timing and efficiency of applications. Currently, bactericide applications are initiated based upon crop phenology (33). However, the time of appearance of symptoms of Xanthomonas leaf blight varies among fields, and growers may make more applications than needed to manage the disease effectively. Schwartz et al. (34) developed multiple regression models to predict the appearance and severity of Xanthomonas leaf blight in southern Colorado. The use of these models to time initiation of bactericide applications may reduce the number of bactericide applications made without reducing control of the disease. In addition, EBDC fungicides may not be necessary for management of Xanthomonas leaf blight since copper-tolerant strains of *X. axonopodis* pv. *allii* are not prevalent in Colorado (8).

Novel chemical and biological control agents appear promising for reducing the use of conventional bactericides on onion. Paulraj and O'Garro (23) evaluated *Pantoea agglomerans* in growth chamber assays as a biological control agent for Xanthomonas leaf blight of onion and found prophylactic applications of the bacterium to onion foliage greatly reduced severity of disease. Systemic acquired resistance (SAR) can be induced by products such as

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acibenzolar-*S*-methyl (Actigard 50WG, Syngenta Crop Protection, Greensboro, NC), a structural analog of salicylic acid (9). Acibenzolar-*S*-methyl can be an effective alternative to many bactericides and fungicides for control of a number of diseases (1,2,6,9,20,24). Resistance to acibenzolar-*S*-methyl has not been reported for any pathogen, and this product suppressed bacterial spot and speck of tomato caused by copper-tolerant strains of *X. axonopodis* pv. *vesicatoria* and *Pseudomonas syringae* pv. *tomato*, respectively (18). However, acibenzolar-*S*-methyl and other inducers of SAR must be used carefully, as phytotoxicity and yield depression have been documented on some hosts or genotypes treated with SARs (6,26), and its use may aggravate other pest problems (36,37). Acibenzolar-*S*-methyl has not been evaluated for suppression of Xanthomonas leaf blight of onion.

Despite the potential for reducing the amount of copper bactericides and EBDC fungicides to manage Xanthomonas leaf blight in Colorado, no field studies have evaluated the integrated use of disease forecasting, rates of application of EBDC fungicides, and novel chemical and biological controls for management of this disease. New management strategies for Xanthomonas leaf blight are necessary to improve disease control, minimize environmental impact from pesticide applications, and reduce production costs. This series of studies was launched to investigate potential methods for reducing the number of applications of copper bactericides and EBDC fungicides for management of Xanthomonas leaf blight by improving the timing of bactericide applications, and identifying novel chemical treatments and biological control agents with efficacy against *X. axonopodis* pv. *allii*.

MATERIALS AND METHODS

Bacterial strains and culture. A rifampicin resistant mutant of *X. axonopodis* pv. *allii* strain O177 (ATCC 508) was generated as described by Weller and Saettler (39), and is referred to as strain R-O177. Strain R-O177 was resistant to rifampicin at >200 µg/ml, but selection routinely was performed on nutrient agar amended with 50 µg/ml. For growth chamber studies, inoculum of strain R-O177 was cultured by inoculating a single colony of *X. axonopodis* pv. *allii* into 3 ml of nutrient broth in 15-ml culture tubes and incubating at 26°C with vigorous shaking (250 oscillations per min) for 24 h. The bacterial cells were collected by centrifugation at 10,000 × *g* for 5 min before adjusting to approximately 10^5 CFU/ml in sterile magnesium phosphate buffer (0.01 M magnesium sulfate and 0.01 M potassium phosphate, pH 7.2). The buffer was prepared from magnesium sulfate formulated as $MgSO_4 \cdot 7H_2O$ and potassium phosphate derived from a mixture of both dibasic (K_2HPO_4) and monobasic (KH_2PO_4) forms. To prepare inoculum of strain R-O177 for field studies, loopsful of bacteria were streaked onto rifampicin-amended nutrient agar before incubating culture plates at 29°C for 72 h in the dark. Cells were harvested by flooding plates with deionized water and gently scraping the plates with a flame-sterilized spatula. The cell suspension was measured and adjusted spectrophotometrically to 10^8 CFU/ml (OD_{600} = 0.12), and then diluted further to the desired concentration for individual experiments. The bacterial strain was preserved in 15% nutrient glycerol broth at –80°C.

Epiphytic population assays. Experiments were conducted in a growth chamber to determine the effect of acibenzolar-*S*-methyl (Actigard 50WG) and copper hydroxide-mancozeb (ManKocide, Griffin L.L.C., Valdosta, GA) on epiphytic multiplication of *X. axonopodis* pv. *allii* strain R-O177 on onion. Plants of onion cultivars Cometa and Vantage were grown under greenhouse conditions (approximately 24/20°C with a 14-h photoperiod by day and approximately 2 h of supplemental incandescent lighting) until they were 6 to 8 weeks old. For each cultivar, three seeds were planted into a 1-liter pot per experimental unit in MetroMix 200 potting soil (Grace Sierra Horticultural Products Co., Milpitas, CA). Plants treated with Actigard 50WG were sprayed with 35 g a.i./ha amended with 0.25 vol/vol Latron AG-98 (Dow AgroSciences, Indianapolis, IN) in 90 liters of water per hectare in a spray chamber pressurized to 275 kPa using compressed CO_2. Plants were similarly treated with copper hydroxide-mancozeb at 1.03 kg a.i. copper hydroxide + 0.34 kg a.i. mancozeb per hectare amended with 0.25% vol/vol Latron AG-98 1 h prior to inoculation. Nontreated plants were sprayed with water amended with 0.25% vol/vol Latron AG-98.

Twenty pots planted with each cultivar were inoculated by spraying the plants to runoff with a suspension of *X. axonopodis* pv. *allii* (10^5 CFU/ml) using a Crown SpraTool (Aerovoe Industries, Inc., Gardnerville, NV). After inoculation, plants were left to air-dry for approximately 15 min and then sampled by removing all aboveground plant material from the plants in each of four pots per cultivar, and placing the plants into individual plastic bags. The remainder of the plants were placed in a growth chamber and incubated at 28/24°C day/night at a light intensity of 350 $\mu M \cdot s^{-1} \cdot m^{-2}$ and 100% relative humidity. The plants were misted daily with tap water to runoff with a spray bottle. Four pots of each cultivar were sampled destructively each day for 4 days after inoculation. An experimental unit consisted of one pot of a given cultivar with three plants sampled destructively each day. The experiment was designed as a completely randomized design with four replications.

At each sampling, harvested plants were weighed and placed into sterile 250-ml flasks containing 100 ml of magnesium phosphate buffer, and shaken at 250 oscillations per min for 60 min at room temperature (approximately 22°C). Aliquots (100 µl) were diluted in 10-fold serial dilutions in sterile magnesium phosphate buffer, and plated onto two nutrient agar plates per dilution amended with rifampicin and cycloheximide at 50 µg/ml. Colonies of *X. axonopodis* pv. *allii* were enumerated after 72 h of incubation at 29°C. A subset of rifampicin-resistant colonies were confirmed as *X. axonopodis* pv. *allii* by standard physiological and biochemical tests (30), including Gram stain reaction, pigmentation on yeast dextrose carbonate medium, fluorescence on King's medium B, indole test, growth on 0.1% tetrazolium chloride, oxidase test, starch hydrolysis, oxidative utilization of glucose, catalase test, production of H_2S from cysteine, presence of arginine dihydrolase, and casein hydrolysis test. The experiment was repeated once.

In planta population assays. Multiplication of *X. axonopodis* pv. *allii* strain R-O177 in onion plants was quantified as previously described by Gent et al. (8). Plants were treated with acibenzolar-*S*-methyl (Actigard 50WG), copper hydroxide-mancozeb (ManKocide), or water as described above. The youngest fully extended leaf of an 8-week-old onion plant of cultivars Cometa and Vantage was pinpricked in seven locations along the leaf at 1-cm intervals, using a 22 gauge needle bearing strain R-O177 removed from 72-h-old colonies on nutrient agar. Each pinpricked leaf area was inoculated with a bacterial droplet approximately equal in size to the needle tip. One-centimeter-long inoculated leaf sections were removed from one plant per cultivar per day for 7 days and vortexed in 5 ml of sterile magnesium phosphate buffer amended with 0.25% vol/vol X-77 nonionic surfactant (Helena Chemical Co., Fresno, CA) to remove copper hydroxide-mancozeb residues on the leaf surface. The leaf was surface-disinfested in 95% ethanol for 30 s, and then rinsed three times in sterile magnesium phosphate buffer. The leaf was then ground aseptically in 1 ml of sterile magnesium phosphate buffer using a mortar and pestle. The homogenate was diluted serially and plated onto nutrient agar amended with rifampicin and cycloheximide at 50 µg/ml. Rifampicin-resistant colonies were enumerated after 72 h of incubation at 29°C, and representative colonies were confirmed as strain R-O177 by physiological and biochemical tests as described above. The experiment was a completely randomized design with four replications. An experimental unit was considered an individual leaf section re-

moved from a plant on a given day. The entire experiment was repeated once.

Evaluation of acibenzolar-*S*-methyl and biological control agents. Field plots were established from 2002 to 2004 near Fort Collins, CO, at the Colorado State University Agricultural Research, Development, and Education Center; near Rocky Ford, CO, at the Arkansas Valley Research Center; and near Yuma, CO, at the Irrigation Research Foundation to evaluate acibenzolar-*S*-methyl and biological control agents for suppression of Xanthomonas leaf blight. Plots at Fort Collins (2002 to 2004) and Yuma (2003 and 2004) were established using the yellow onion cultivar Vantage from seed planted approximately 0.10 m apart in beds on 0.76-m centers. Each bed contained one (Yuma) or two (Fort Collins) rows spaced 0.15 m apart. At Rocky Ford (2002 to 2004), seeds of the yellow onion cultivar X-201 were planted approximately 0.10 m apart in beds on 1.06-m centers. Each bed contained two rows spaced 0.15 m apart. Fields at Fort Collins and Rocky Ford were furrow irrigated, but irrigation was provided by center-pivot in fields at Yuma. Fertilizer, herbicides, and insecticides were applied according to standard production practices (32). Each plot consisted of one bed 1.06 m (Rocky Ford) or 0.76 m (Fort Collins and Yuma) wide by 4.6 m long separated from adjacent beds by a single nontreated row. Treatments were applied with a CO_2-pressurized backpack sprayer and two TeeJet 8002 flat fan nozzles (Spraying Systems Co., Wheaton, IL) spaced equally on a 0.45-m-wide boom, in an application volume of 234 liters/ha.

The same treatments were used at all locations during a given year, but varied among years. Each experiment was arranged in a randomized split-block design with four replications. Whole plot treatments consisted of applications of chemical treatments or biological control agents (nontreated, copper hydroxide [Kocide 2000, Griffin L.L.C.], acibenzolar-*S*-methyl, *Pantoea agglomerans* [BlightBan C9-1, Nufarm, Burr Ridge, IL], or *P. agglomerans* and *Pseudomonas fluorescens* [BlightBan A506/C9-1, Nufarm]), and the subplot treatments consisted of the presence or absence of weekly copper hydroxide-mancozeb (ManKocide) applications. The untreated plots were not treated with water. Plots were monitored weekly to determine the date of initial appearance of symptoms and subsequent development of Xanthomonas leaf blight. Average percent disease severity was estimated for each plot using a modified Horsfall-Barratt scale (11) and converted to a percentage. Weekly disease severity ratings were used to calculate the relative area under the disease progress curve (RAUDPC) using the formula

$$\{\Sigma_{i=1}^{n}[(x_{i+1} + x_i)/2](t_{i+1} - t_i)\}/(t_n - t_i)$$

where x_i = disease severity at time t_i. At maturity, a 3-m subsection of each subplot was topped mechanically and harvested, graded, and weighed to estimate bulb yields.

In 2002, plots were established near Fort Collins and Rocky Ford. Treatments consisted of 10 weekly applications of copper hydroxide (Kocide 2000) at 0.90 kg a.i./ha or acibenzolar-*S*-methyl (Actigard 50WG) at 35 g a.i./ha, with or without copper hydroxide-mancozeb (ManKocide) at 1.03 kg a.i. copper hydroxide + 0.34 kg a.i. mancozeb per hectare (later applied as subplot). The first application was made 76 and 108 days after planting at Fort Collins and Rocky Ford, respectively. Nontreated rows separating treated plots were inoculated with 10^8 CFU of *X. axonopodis* pv. *allii* strain R-O177 per milliliter of water 113, 118, and 127 days after planting at Fort Collins, and 151 and 164 days after planting at Rocky Ford, to initiate an epidemic of Xanthomonas leaf blight.

In 2003, experiments were conducted near Rocky Ford, Fort Collins, and Yuma. Treatments consisted of 9 or 10 applications of copper hydroxide (Kocide 2000) at 0.90 kg a.i./ha or acibenzolar-*S*-methyl (Actigard 50WG) at 35 g a.i./ha with or without copper hydroxide-mancozeb (ManKocide) at 1.03 kg a.i. copper hydroxide + 0.34 kg a.i. mancozeb per hectare. Copper hydroxide and copper hydroxide-mancozeb treatments were applied weekly beginning 99, 105, and 97 days after planting at Fort Collins, Rocky Ford, and Yuma, respectively. Ten applications were made at Rocky Ford and Yuma, and 9 applications were made at Fort Collins. Four weekly acibenzolar-*S*-methyl applications were made at each location in 2003, beginning 99, 105, or 97 days after planting at Fort Collins, Rocky Ford, and Yuma, respectively. Plots were inoculated with 10^8 CFU of *X. axonopodis* pv. *allii* strain R-O177 per milliliter of water 133, 140, and 144 days after planting at Fort Collins, 124, 136, 143, 150, and 157 days after planting at Rocky Ford, and 126 and 133 days after planting at Yuma.

In 2004, the experiments were repeated at the same three locations as in 2003. Treatments and rates of application were as described for the 2003 trials, but in addition BlightBan C9-1 (lyophilized cells of *Pantoea agglomerans* strain C9-1) and BlightBan C9-1/A506 (equal mixture of lyophilized cells of *Pantoea agglomerans* strain C9-1 and *Pseudomonas fluorescens* strain A506) were included. BlightBan C9-1 was applied at 10^{10} CFU of *Pantoea agglomerans* strain C9-1 per milliliter, and BlightBan C9-1/A506 was applied at 5×10^9 CFU each of *Pantoea agglomerans* strain C9-1 and *Pseudomonas fluorescens* strain A506 per milliliter of water. Weekly applications of each treatment were initiated 101, 101, and 98 days after planting at Fort Collins, Rocky Ford, and Yuma, respectively. A total of 9, 11, and 12 copper hydroxide applications were made at Fort Collins, Rocky Ford, and Yuma, respectively. Four weekly applications of acibenzolar-*S*-methyl and two weekly applications of the biological control agents were made beginning on the same day as the copper hydroxide treatments. Plots were inoculated with 10^8 CFU of *X. axonopodis* pv. *allii* strain R-O177 per milliliter of water 114 and 117 days after planting in Fort Collins, 122 days after planting in Rocky Ford, and 152 and 166 days after planting in Yuma.

Identification of the critical period for bactericide applications. Experiments were established at the Arkansas Valley Research Center near Rocky Ford from 2002 to 2004 to determine the critical period for Xanthomonas leaf blight control using copper bactericides. Each plot consisted of a 7.5-m length of one bed planted with two lines of the onion cultivar X-201. Plots were arranged in a randomized split-block design with three replications, with the timing of the first copper hydroxide application as the whole-plot treatment and the rate of EBDC fungicide as the subplot treatment.

To determine the critical period for bactericide applications, the timing of the first copper hydroxide (Kocide 2000) application ranged from 4 weeks before to 2 weeks after bulb initiation. Whole plots received a weekly application of 0.90 kg a.i. copper hydroxide per hectare, beginning approximately 4 weeks pre-bulb initiation to 2 weeks post-bulb initiation. Subplots were treated with maneb (Maneb 75DF) at a rate of 0, 0.42, 0.84, or 1.68 kg a.i./ha initiated on the same day as the copper hydroxide treatment for each whole-plot treatment. The control plots were not treated with copper hydroxide, but were treated with maneb at 0, 0.42, 0.84, or 1.68 kg a.i./ha. Copper hydroxide and maneb applications were repeated weekly until approximately 14 days before harvest. In 2002, the 4-week pre-bulb initiation, 3-week pre-bulb initiation, 2-week pre-bulb initiation, 1-week pre-bulb initiation, bulb initiation, 1-week post-bulb initiation, and 2-week post-bulb initiation spray programs were started 101, 108, 115, 123, 130, 137, and 144 days after planting, respectively. The plots received 9, 8, 7, 6, 5, 4, and 3 total applications, respectively. The control plots received 5 applications of maneb at the rates specified previously, beginning 130 days after planting. In 2003, copper hydroxide treatments were started 92, 96, 105, 114, 122, 128, and 135 days after planting for the sequential spray programs with 9, 8, 7, 6, 5, 4, and 3 total applications made, respectively. The control plots received 5 applications of Maneb 75DF at the rates specified above, beginning 122 days after planting. In 2004, copper hydroxide treatments were started 71, 77, 84, 93, 98, 106, and 112 days after

planting, with 14, 13, 12, 11, 10, 9, and 8 total applications made for the sequential spray programs, respectively. The control plots received 10 applications of maneb at the rates specified above, beginning 98 days after planting. All treatments were applied in 90 liters of water per hectare using a 45-cm boom equipped with two 8002 even flat-fan nozzles (TeeJet, Spraying Systems Company, Sioux Falls, SD) and pressurized to 275 kPa using compressed CO_2. Plots were not inoculated with *X. axonopodis* pv. *allii*.

Statistical analyses. Statistical analyses were performed using the PROC MIXED function of SAS version 9.1 (SAS Institute, Cary, NC). Bacterial population data from growth chamber studies were log transformed to achieve independently and normally distributed experimental errors with a common variance. The area under the bacterial growth curve was calculated from growth chamber studies with epiphytic and in planta populations of *X. axonopodis* pv. *allii* and used as the response variable for statistical analyses. Replications of the entire experiment were considered fixed factors in preliminary analysis of epiphytic and in planta population assays, but were not significant in the models (P = 0.4214 and P = 0.0566, respectively). Therefore, data from each replication of the experiments were pooled, and in subsequent analyses replications of experimental units and entire experiments were considered random factors. In field studies, locations and replications were considered fixed and random factors, respectively, in the mixed model analyses. RAUDPC was used as the response variable in field studies.

RESULTS

Epiphytic population assays. The mean and standard deviation (SD) for the area under the bacterial population growth curve combined over two replications of the experiment were 18.1 (SD = 1.4), 17.5 (SD = 0.4), and 22.7 (SD = 0.6) for acibenzolar-*S*-methyl, copper hydroxide-mancozeb, and untreated control plants, respectively. Epiphytic populations of *X. axonopodis* pv. *allii*, as measured by the area under the bacterial population growth curve, were similar for cultivars Vantage and Cometa (P = 0.088, data not shown). Cultivar by treatment interactions were not significant for total epiphytic populations (P = 0.204). However, *X. axonopodis* pv. *allii* populations on plants treated with acibenzolar-*S*-methyl or copper hydroxide-mancozeb differed from those on nontreated plants among sampling days. The area under the bacterial growth curve was not different among these three treatments (P = 0.089, data not shown) (Fig. 1). Both acibenzolar-*S*-methyl and copper hydroxide-mancozeb reduced epiphytic populations of *X. axonopodis* pv. *allii* by 0.5 to 2.0 logarithmic units per gram on leaf tissue (fresh weight) at each sample period 24 h after inoculation compared with nontreated plants.

In planta population dynamics. The mean and standard deviation for the area under the bacterial population growth curve combined over two replications of the experiment were 43.3 (SD = 2.4), 43.6 (SD = 2.3), and 45.8 (SD = 2.1) for acibenzolar-*S*-methyl, copper hydroxide-mancozeb, and nontreated plants, respectively. In planta populations of *X. axonopodis* pv. *allii* in leaves of onion cultivars Vantage and Cometa were similar as measured by the area under the bacterial population growth curve (P = 0.334, data not shown). Cultivar by treatment interactions were not significant for total in planta populations of the pathogen across all five sampling days (P = 0.137). Treatment with acibenzolar-*S*-methyl or copper hydroxide-mancozeb reduced in planta bacterial populations compared with nontreated plants (Fig. 2). *X. axonopodis* pv. *allii* populations were reduced on acibenzolar-*S*-methyl or copper hydroxide-mancozeb plants for five of six sampling days compared with the nontreated plants. However, this decrease was less than 1 logarithmic unit per gram fresh weight of onion tissue.

Evaluation of acibenzolar-*S*-methyl and biological control agents. Despite repeated inoculations with *X. axonopodis* pv. *allii* in 2002, an epidemic of Xanthomonas leaf blight did not occur at either location. However, the lack of disease development allowed determination of the impact of foliar applications of acibenzolar-*S*-methyl on onion yield and grade in the absence of disease. Ten weekly applications of acibenzolar-*S*-methyl (2.5 times as many applications as recommended on the Actigard 50WG label for registered crops) reduced total onion yield by 8.7 (27%) and 9.3 t/ha (22%) at Fort Collins and Rocky Ford, respectively (Table 1), but did not have an effect on yield of medium (both sites) or jumbo (Rocky Ford) grade bulbs.

In 2003, applications of acibenzolar-*S*-methyl controlled Xanthomonas leaf blight as effectively (Fort Collins and Yuma) or better than (Rocky Ford) copper hydroxide and copper hydroxide-mancozeb applications (Table 2). At the Rocky Ford site, acibenzolar-*S*-methyl applications reduced the RAUDPC 43% compared with the copper hydroxide-mancozeb applications. At all locations, acibenzolar-*S*-methyl applied alone was as effective as acibenzolar-*S*-methyl applied in combination with copper hydroxide-mancozeb. Acibenzolar-*S*-methyl applications increased the yield of jumbo grade bulbs at Rocky Ford compared with copper hydroxide-mancozeb applications and nontreated plots, but a combination of acibenzolar-*S*-methyl and copper hydroxide-mancozeb

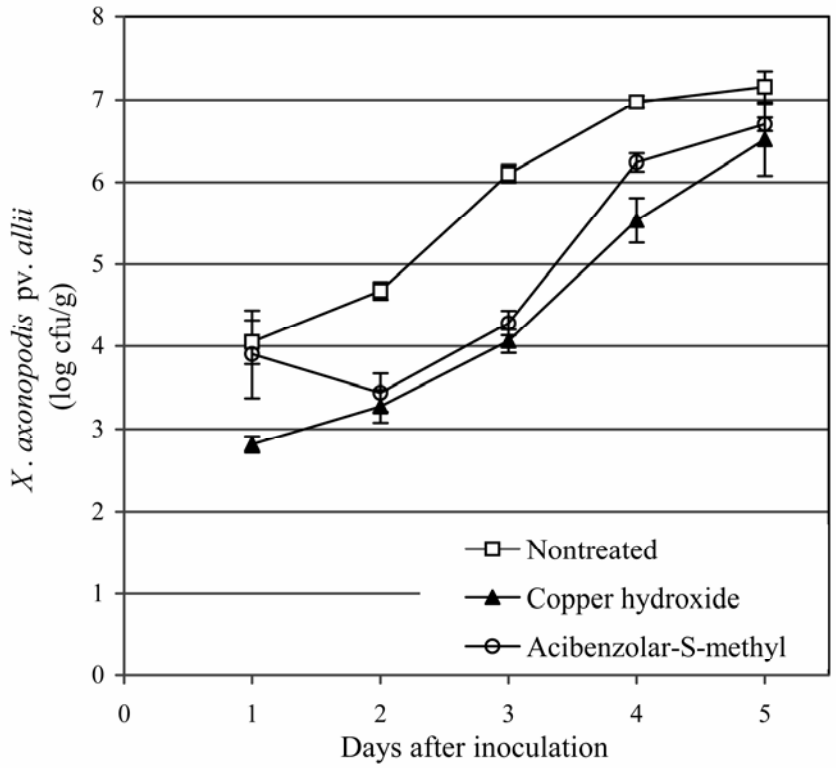

Fig. 1. Epiphytic multiplication of *Xanthomonas axonopodis* pv. *allii* on onion treated with copper hydroxide, acibenzolar-*S*-methyl, or water (nontreated control). Onion cultivars Vantage and Cometa were used in these growth chamber studies, but epiphytic populations of *X. axonopodis* pv. *allii* did not differ among cultivars (P = 0.088 for cultivars in the analysis of variance). Data are means of four replications averaged over cultivars and two replications of the entire experiment (n = 16) ± standard error of the mean.

reduced jumbo yield 24% compared with acibenzolar-*S*-methyl applications alone at this site. Total yields at the three locations were not affected by acibenzolar-*S*-methyl or copper bactericide applications compared with nontreated plots.

In 2004, applications of acibenzolar-*S*-methyl controlled Xanthomonas leaf blight as effectively as (Rocky Ford and Yuma sites) or better than (Fort Collins site) applications of copper hydroxide and copper hydroxide-mancozeb (Table 3). At Fort Collins, RAUDPC was reduced 29 and 33% for plots treated with acibenzolar-*S*-methyl compared with copper hydroxide or copper hydroxide-mancozeb, respectively. At all locations, acibenzolar-*S*-methyl applications alone were as effective as combination applications with copper hydroxide-mancozeb. Bulb yields did not differ among treatments at any location.

The application of *Pantoea agglomerans* strain C9-1 + *Pseudomonas fluorescens* strain A506 was as effective as copper hydroxide (at all sites) or copper hydroxide-mancozeb (Rocky Ford and Fort Collins) in reducing severity of Xanthomonas leaf blight compared with nontreated plots (Table 3). The combination application of these biological control agents with copper hydroxide-mancozeb at Yuma provided better disease control than the biological control agents alone, and was as effective as applications of copper hydroxide-mancozeb alone. At all locations, disease severity in plots treated with *Pantoea agglomerans* strain C9-1 did not differ from that in nontreated plots.

Identification of the critical period for bactericide applications. Natural epidemics of Xanthomonas leaf blight occurred in 2003 and 2004, but the disease did not develop in 2002, so comparisons among treatments were not possible in 2002. Yields were not expected to differ among treatments in the absence of disease, so plots were not harvested in 2002. In 2003, a late-season (within 14 days of harvest) epidemic of Xanthomonas leaf blight occurred, and all spray programs that included copper hydroxide reduced the RAUDPC compared with the nontreated plots (Table 4). Plots in which spray programs were started 2 to 4 weeks before bulb initiation had less Xanthomonas leaf blight than those started at bulb initiation or later. Disease severity did not differ among spray programs initiated 4, 2, or 1 week(s) before bulb initiation, but two to three fewer applications of copper hydroxide and maneb were needed to achieve this level of disease suppression when spray programs were started 1 to 2 weeks versus 3 to 4 weeks before bulb initiation. The rate of maneb application did not affect disease severity. Yields were not estimated in 2003 because disease pressure was not considered severe enough to cause measurable yield effects.

In 2004, the epidemic of Xanthomonas leaf blight started earlier than in 2003, lasting 27 days to harvest. However, there were no significant differences in severity of Xanthomonas leaf blight or bulb yields among any of the treatments (Table 4).

DISCUSSION

Applications of copper bactericides tank-mixed with EBDC fungicides are a central component of onion production in Colorado for control of Xanthomonas leaf blight and other bacterial diseases (33), but this strategy for bacterial disease management increases production costs and reliance upon EBDC fungicides. A critical need exists for new disease management strategies that are more effective, economical, and environmentally sound than calendar-based copper/EBDC bactericide spray programs. In this study, we demonstrated that acibenzolar-*S*-methyl and biological control agents can be used to control Xanthomonas leaf blight as effectively as copper-based spray programs, and may potentially replace bactericides currently used to suppress this disease in Colorado. Additionally, under the conditions of this study, the disease was managed effectively without early-season (prior to 1 or 2 weeks before bulb initiation) copper applications or maneb amendments to copper applications.

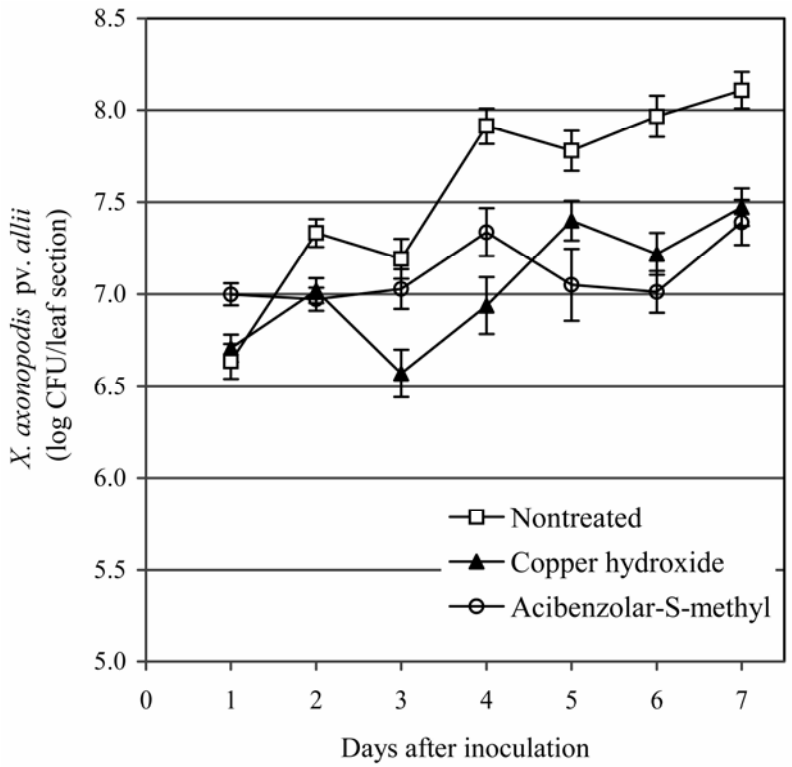

Fig. 2. In planta multiplication of *Xanthomonas axonopodis* pv. *allii* in onion in relation to treatment with copper hydroxide, acibenzolar-*S*-methyl, or water (control). Onion cultivars Vantage and Cometa were used in these growth chamber studies, but in planta populations of *X. axonopodis* pv. *allii* did not differ among cultivars ($P = 0.334$ for cultivars in the analysis of variance). Data are means of four replications averaged over cultivars and two replications of the entire experiment ($n = 16$) ± standard error of the mean.

Table 1. Effect of copper hydroxide and acibenzolar-*S*-methyl on onion bulb yield in the absence of Xanthomonas leaf blight at two locations in Colorado in 2002

	Yield (t/ha, total and by bulb size)[x]				
	Rocky Ford[y]			Fort Collins[y]	
Treatment[z]	Medium	Jumbo	Total	Medium	Total
Control	26.1 a	8.3 a	42.7 a	15.4 a	31.8 a
Copper hydroxide	22.8 a	6.5 a	39.9 ab	9.2 a	28.7 ab
Acibenzolar-*S*-methyl	20.8 a	5.6 a	33.4 b	10.9 a	23.1 b

[x] Treatments within a column followed by the same letter are not significantly different based upon Fisher's protected least significant difference ($\alpha = 0.05$). Data are means of four replications.

[y] Cultivar X-201 was used at Rocky Ford, and cultivar Vantage was used at Fort Collins. Medium grade bulbs are those with a diameter of 5.1 to 8.3 cm. Jumbo grade bulbs are those with a diameter of 8.3 to 9.5 cm. Total is the yield of all bulbs. Jumbo grade bulbs were not produced at Fort Collins.

[z] Ten weekly applications of copper hydroxide and acibenzolar-*S*-methyl were applied to plots beginning approximately 2 weeks before bulb initiation.

Acibenzolar-*S*-methyl has potential as a valuable tool for management of Xanthomonas leaf blight in Colorado. In growth chamber studies, applications of acibenzolar-*S*-methyl reduced in planta and epiphytic populations of *X. axonopodis* pv. *allii* populations for two onion cultivars as effectively as copper hydroxide-mancozeb applications. Under field conditions in which plants were inoculated with a copper-sensitive strain of *X. axonopodis* pv. *allii*, four weekly applications of acibenzolar-*S*-methyl at 35 g a.i./ha were equally or more effective than 9 to 10 weekly applications of either copper hydroxide or copper hydroxide-mancozeb, and had no negative effect on bulb yield or grade when Xanthomonas leaf blight was present. In six experiments, disease suppression was not improved by combining acibenzolar-*S*-methyl with copper hydroxide-mancozeb applications, suggesting that this novel chemical treatment may replace copper hydroxide-mancozeb applications for management of Xanthomonas leaf blight of onion.

Disease severity in our plots was comparable to that observed under natural

Table 2. Severity of Xanthomonas leaf blight and yield of onion bulbs in relation to copper bactericide and acibenzolar-*S*-methyl applications at three locations in Colorado in 2003

	Location, disease severity, and yield (t/ha, total and by bulb size)[w,x]							
	Rocky Ford[x]				Fort Collins[y]		Yuma[y]	
Whole plot treatment[z]	RAUDPC	Medium	Jumbo	Total	RAUDPC	Total	RAUDPC	Total
Control	0.17 a	10.6 ab	12.1 a	24.4 a	0.25 a	7.8 a	0.37 a	7.3 a
Copper hydroxide	0.15 b	8.3 ab	16.1 abc	25.7 ab	0.18 b	9.3 a	0.24 ab	7.7 a
Acibenzolar-*S*-methyl	0.08 c	11.1 bc	20.0 c	32.1 ab	0.15 b	9.0 a	0.16 b	6.4 a
Copper hydroxide-mancozeb subplot								
Control	0.14 b	16.1 c	15.7 b	33.8 b	0.18 b	7.3 a	0.28 ab	6.8 a
Copper hydroxide	0.14 b	4.6 a	17.1 abc	22.2 ab	0.14 b	8.7 a	0.25 ab	7.7 a
Acibenzolar-*S*-methyl	0.09 c	12.3 bc	15.3 ab	29.5 ab	0.14 b	8.5 a	0.18 b	6.8 a
ANOVA factor[z]								
Whole plot treatment	0.0022	0.0597	0.5732	0.4616	0.0970	0.0784	0.2913	0.6331
Subplot treatment	0.3033	0.3972	0.9504	0.4494	0.0989	0.4464	0.1331	0.9513
Whole plot*subplot	0.0774	0.0290	0.0048	0.0097	0.0178	0.8957	0.0120	0.3627

[w] RAUDPC = relative area under the disease progress curve. RAUDPC was calculated as $\{\Sigma^{n}_{i=1}[(x_{i+1}+x_i)/2](t_{i+1}-t_i)\}/(t_n-t_i)$ where x_i is disease severity at time t_i. Treatments within a column followed by the same letter are not significantly different based on an F protected least significant difference (α = 0.05). Data are means of four replications.

[x] Cultivar X-201 was used at Rocky Ford, and cultivar Vantage was used at Fort Collins and Yuma. Medium grade bulbs are those with a diameter of 5.1 to 8.3 cm. Jumbo grade bulbs are those with a diameter of 8.3 to 9.5 cm. Total is the yield of all bulbs.

[y] Total yield at Fort Collins and Yuma (kg) of 10 bulbs harvested randomly from each plot. Yield at Rocky Ford was estimated by harvesting all bulbs from a 3-m section of one bed.

[z] Ten weekly applications of copper hydroxide, copper hydroxide-mancozeb, or four weekly applications of acibenzolar-*S*-methyl were applied to plots beginning approximately 2 weeks before bulb initiation. The experiment was arranged as a randomized split-block design, where whole plot treatments included nontreated control, copper hydroxide, and acibenzolar-*S*-methyl applications, and the subplot treatments included the presence or absence of weekly applications of copper hydroxide-mancozeb. Values are probabilities for each factor in the ANOVA.

Table 3. Severity of Xanthomonas leaf blight and yield of onion bulbs in relation to copper bactericide, acibenzolar-*S*-methyl, and biological control agents applied at each of three locations in Colorado in 2004

	Location, disease severity, and yield (t/ha, total and by bulb size)[x,y]										
	Rocky Ford				Fort Collins			Yuma			
Whole plot treatment[z]	RAUDPC	Medium	Jumbo	Total	RAUDPC	Medium	Total	RAUDPC	Medium	Jumbo	Total
Control	0.48 a	28.2 a	16.0 a	47.4 a	0.29 a	29.3 a	39.5 a	0.06 a	15.0 a	14.8 a	32.7 a
Copper hydroxide	0.29 bc	23.6 a	14.7 a	41.9 a	0.21 b	28.1 a	36.9 a	0.03 bc	16.1 a	15.5 a	34.9 a
Acibenzolar-*S*-methyl	0.23 c	25.4 a	20.4 a	48.8 a	0.15 d	32.7 a	47.8 a	0.03 bc	14.4 a	16.6 a	33.0 a
Pa C9-1	0.36 a	28.4 a	14.2 a	46.7 a	0.30 a	26.1 a	39.0 a	0.04 ab	14.6 a	19.0 a	35.5 a
Pa C9-1/Pf A506	0.26 bc	25.2 a	14.0 a	42.8 a	0.22 bc	30.0 a	39.5 a	0.04 b	13.7 a	15.6 a	31.1 a
Copper hydroxide-mancozeb subplot											
Control	0.34 b	27.0 a	17.4 a	48.8 a	0.24 ab	24.2 a	35.9 a	0.03 c	11.9 a	20.8 a	34.7 a
Copper hydroxide	0.24 c	23.8 a	16.5 a	43.7 a	0.20 bc	24.2 a	41.2 a	0.02 c	11.3 a	20.0 a	33.9 a
Acibenzolar-*S*-methyl	0.21 c	25.0 a	19.7 a	47.9 a	0.15 d	29.3 a	41.7 a	0.02 c	12.1 a	16.7 a	31.5 a
Pa C9-1	0.31 bc	28.2 a	10.8 a	45.1 a	0.23 bc	30.0 a	39.3 a	0.02 c	11.1 a	21.9 a	36.2 a
Pa C9-1/Pf A506	0.21 c	27.9 a	15.8 a	44.9 a	0.19 cd	33.4 a	41.2 a	0.02 c	12.0 a	15.4 a	29.3 a
ANOVA factor[z]											
Whole plot	0.0026	0.5102	0.2426	0.3507	0.0001	0.6406	0.4219	0.1115	0.8658	0.4261	0.1498
Subplot	0.0129	0.8997	0.9241	0.6775	0.0552	0.4122	0.7093	0.0065	0.1286	0.2742	0.9125
Whole plot*subplot	0.0055	0.9672	0.8626	0.8474	0.2517	0.0837	0.1930	0.0090	0.4655	0.5929	0.8886

[x] RAUDPC = relative area under the disease progress curve. RAUDPC was calculated as $\{\Sigma^{n}_{i=1}[(x_{i+1}+x_i)/2](t_{i+1}-t_i)\}/(t_n-t_i)$ where x_i is disease severity at time t_i. Treatments within a column followed by the same letter are not significantly different based on an F protected least significant difference (α = 0.05). Data are means of four replications.

[y] Cultivar X-201 was used at Rocky Ford, and cultivar Vantage was used at Fort Collins and Yuma. Medium grade bulbs are those with a diameter of 5.1 to 8.3 cm. Jumbo grade bulbs are those with a diameter of 8.3 to 9.5 cm. Total is the yield of all bulbs.

[z] Ten weekly applications of copper hydroxide, copper hydroxide-mancozeb, four weekly applications of acibenzolar-*S*-methyl, or two weekly applications of *Pantoea agglomerans* strain C9-1 (Pa C9-1) or *P. agglomerans* strain C9-1 + *Pseudomonas fluorescens* strain A506 (Pa C9-1/Pf A506) were applied beginning approximately 2 weeks before bulb initiation. The experiment was arranged as a randomized split-block design, where whole plot treatments included nontreated control, copper hydroxide, acibenzolar-*S*-methyl, *Pantoea agglomerans* strain C9-1, or *P. agglomerans* strain C9-1 + *Pseudomonas fluorescens* strain A506 treatments, and subplot treatments included the presence or absence of weekly applications of copper hydroxide-mancozeb. Values are probabilities for each factor in the ANOVA.

epidemics in Colorado (34), but may not be representative of the severity of Xanthomonas leaf blight epidemics in tropical or subtropical environments. In Barbados, epiphytic populations of *X. axonopodis* pv. *allii* are often present on onion seedlings, and disease epidemics may develop shortly after emergence (21–23). Disease appearance in Colorado and the Central High Plains appears to be closely associated with the initiation of reproductive development, and weather conditions during vegetative growth are not associated with the appearance or severity of Xanthomonas leaf blight (34). However, disease appearance is associated strongly with rainfall and temperature near and after bulb initiation in Colorado (34), and we inoculated plants at or shortly after bulb initiation to mimic natural epidemic development in this environment. This experimental approach demonstrated the effectiveness of applications of acibenzolar-*S*-methyl for managing Xanthomonas leaf blight on long-day onion cultivars in this semi-arid environment, but the timing and effectiveness of the treatments investigated in this study may need to be validated in other onion production environments before being adopted for management of Xanthomonas leaf blight.

Phytotoxicity has been reported with applications of acibenzolar-*S*-methyl on tobacco (6), tomato transplants (17), and pepper (26), and this compound must be used carefully to avoid phytotoxicity to onion. A significant reduction in total yield was observed when 10 weekly applications of acibenzolar-*S*-methyl were made in the absence of disease, but not when four applications were made in the presence of low to high levels of disease pressure. Cole (6) reported that increasing the volume in which acibenzolar-*S*-methyl was applied reduced phytotoxicity of the product to tobacco. In this study, however, acibenzolar-*S*-methyl was evaluated at only one application volume simulating conventional ground-rig application for Colorado onion production. Additional research is needed to evaluate applications of acibenzolar-*S*-methyl at a range of volumes, including volumes typical for aerial and chemigation applications. Although four or more applications of acibenzolar-*S*-methyl were evaluated at one rate in this study, Xanthomonas leaf blight may be effectively controlled at lower application rates or with fewer than four applications. In other studies, Gent and Schwartz (7) demonstrated Xanthomonas leaf blight suppression with two applications of acibenzolar-*S*-methyl combined with six applications of copper hydroxide-mancozeb, and this spray program was more effective than copper hydroxide-mancozeb applications alone.

Two onion cultivars susceptible to Xanthomonas leaf blight (J. M. Lang and H. F. Schwartz, *unpublished data*) were evaluated in field trials with acibenzolar-*S*-methyl in this study. Combining the planting of cultivars with moderate levels of host resistance to Xanthomonas leaf blight with applications of acibenzolar-*S*-methyl may contribute additively to disease control. Tomato genotypes susceptible or resistant to early blight (caused by *Alternaria solani*) were demonstrated to have different accumulation patterns of pathogenesis-related (PR) proteins in response to pathogen challenge and induced systemic resistance (15), and higher constitutive and inducible levels of some PR proteins were associated with quantitative disease resistance. Resistant genotypes were also associated with a more rapid induction of PR genes in the early stages of infection (16). Acibenzolar-*S*-methyl is known to activate a number of physiological, biochemical, and ultrastructural changes in plants (2,9,24). The level of activation in onion plants treated with this product may be greater in onion genotypes with more rapid induction and higher levels of inducible defense pathways. However, applications of acibenzolar-*S*-methyl may also negatively impact some genotypes (26) and should be evaluated on a broad collection of onion cultivars to determine potential interactions between this SAR-inducing compound and plant genotypes under a range of environmental conditions.

Reports of SAR induction by acibenzolar-*S*-methyl against biotrophic and necrotrophic plant pathogens as well as insect pests are increasingly common in the literature (1,2,6,9,17,20,24,26), but acibenzolar-*S*-methyl could potentially aggravate some diseases or insect pests of onion. Interactions between the salicylic and jasmonic acid pathways are not fully understood, but these pathways appear to mediate resistance to distinct pathogens (36). SAR is energetically costly to plants, and other defense pathways are often downregulated in response to a SAR reaction (10,14,36,37), but interactions among defense pathways vary depending on the plant species, the pests attacking the plants, and the physiological state or stress of the plants (25). We have evaluated onion

Table 4. Effect of timing of initiation of weekly copper hydroxide applications and rate of maneb on severity of Xanthomonas leaf blight and bulb yield of the onion cultivar X-201 at Rocky Ford, CO, in 2003 and 2004

	2003		2004				
					Yield (t/ha)[y]		
Whole plot timing treatment[w]	Total no. of applications[x]	RAUDPC[y]	Total no. of applications[y]	RAUDPC	Medium	Jumbo	Total
Control	5	0.06 a	10	0.05 a	10.4 a	41.3 a	52.3 a
4-weeks pre-bulb	9	0.01 c	14	0.04 a	13.6 a	37.3 a	51.5 a
3-weeks pre-bulb	8	0.02 bc	13	0.04 a	11.4 a	41.3 a	52.8 a
2-weeks pre-bulb	7	0.01 c	12	0.05 a	11.7 a	42.8 a	53.9 a
1 week pre-bulb	6	0.02 bc	11	0.04 a	11.8 a	40.9 a	55.3 a
Bulb initiation	5	0.03 b	10	0.05 a	10.9 a	44.2 a	58.7 a
1 week post-bulb	4	0.03 b	9	0.05 a	11.5 a	42.3 a	54.3 a
2 weeks post-bulb	3	0.03 b	8	0.06 a	13.7 a	39.1 a	52.9 a
ANOVA factor[z]							
Whole plot (timing)	…	<0.0001	…	0.0761	0.6199	0.8102	0.4919
Subplot (maneb)	…	0.3755	…	0.8685	0.6565	0.1052	0.0695
Whole plot*subplot	…	0.3250	…	0.3089	0.5476	0.3427	0.4906

[w] Treatments consisted of copper hydroxide applications, where the timing of the first spray was applied at weekly intervals based on crop maturity relative to bulb initiation. Treatments were then applied weekly after each spray program was initiated. The experiment was arranged as a randomized split-block design, where whole plot treatment (timing) was the timing of the first application for each spray program, and subplot treatments were the rates of maneb (applied at 0, 0.42, 0.84, or 1.68 kg a.i./ha).

[x] Control plots were not sprayed with copper hydroxide, but were sprayed with maneb at 0, 0.42, 0.84, or 1.68 kg a.i./ha, at weekly intervals for a total of five applications. Maneb applications on the nontreated control whole plots were started at bulb initiation.

[y] RAUDPC = relative area under the disease progress curve. RAUDPC was calculated as $\{\Sigma^{n}_{i=1}[(x_{i+1}+x_i)/2](t_{i+1}-t_i)\}/(t_n-t_i)$ where x_i is disease severity at time t_i. Treatments within a column followed by the same letter are not significantly different based on an F protected least significant difference (α = 0.05). Data are means of three replications. Medium, jumbo, and total indicate yield of these onion bulb grades.

[z] In the analysis of variance, timing, maneb, and timing*maneb interactions were analyzed using the PROC MIXED procedure of SAS version 9.1. Replications were considered random in the mixed model. Values are probabilities for each factor in the ANOVA.

plants treated with acibenzolar-*S*-methyl for other diseases and pests, and have not observed increases in diseases caused by necrotrophic pathogens (e.g., *Botrytis* spp.) or insects (e.g., *Thrips tabaci*) (data not presented). However, treatment of onion plants with 1 or 10 mM methyl jasmonate triggered induced systemic resistance that reduced populations of *T. tabaci* but also rendered plants hyper-susceptible to Xanthomonas leaf blight (D. H. Gent and H. F. Schwartz, *unpublished data*). Evaluation of acibenzolar-*S*-methyl in a commercial onion production system is necessary for the most effective use of this chemical for management of Xanthomonas leaf blight and other onion diseases.

Biological control of Xanthomonas leaf blight at a level comparable to weekly applications of copper hydroxide-mancozeb was achieved in this study using a commercial formulation of lyophilized cells of a mixture of *Pantoea agglomerans* strain C9-1 and *Pseudomonas fluorescens* strain A506, but not with applications of *Pantoea agglomerans* strain C9-1 alone. A strain of *P. agglomerans* isolated from the onion phyllosphere in Barbados provided nearly complete suppression of Xanthomonas leaf blight in growth chamber assays (23), but no measurable effect of strain C9-1 was observed under field conditions in Colorado in this study. Applications of *Pseudomonas fluorescens* strain A506 alone were not evaluated in this study, so the relative contributions of *Pantoea agglomerans* strain C9-1 and *Pseudomonas fluorescens* strain A506 to Xanthomonas leaf blight suppression remain unclear. Tank-mixes of the biological control agents with copper hydroxide-mancozeb generally did not improve disease suppression compared with the copper hydroxide-mancozeb applications alone, presumably because the bacteria are copper sensitive. Research is needed to evaluate *Pantoea agglomerans* strain C9-1 and *Pseudomonas fluorescens* strain A506 against other bacterial diseases of onion such as center rot (caused by *Pantoea ananatis*) and sour skin (caused by *Burkholderia cepacia*). In petri-dish bioassays, antibiotics produced by *Pantoea agglomerans* strain C9-1 inhibited the growth of *Pantoea ananatis* (D. H. Gent and C. A. Ishimaru, *unpublished data*).

Experiments evaluating the timing of copper hydroxide applications with different rates of maneb tank-mixed with the copper hydroxide indicated that two or more copper hydroxide applications and maneb amendment may be eliminated from a spray program without compromising suppression of Xanthomonas leaf blight when disease pressure is low (2003 and 2004 in this study). Preventative copper hydroxide applications beginning 1 to 2 weeks prior to bulb initiation in 2003 were as effective as spray programs started at the early- to mid-vegetative growth stage (3 to 4 weeks before bulb initiation). In 2004, when a late-season epidemic of Xanthomonas leaf blight occurred, none of the copper hydroxide spray programs significantly improved onion yield or grade relative to the control plots, regardless of the timing of the initial application. Late-season applications of copper hydroxide may not be necessary under low disease pressure because onion yield and grade are unlikely to be affected as plants near maturity.

The critical period for management of Xanthomonas leaf blight using spray programs appears to be near bulb initiation. Schwartz et al. (34) reported no association of weather conditions before bulb initiation with the date of appearance or severity of Xanthomonas leaf blight in Colorado, but copper hydroxide spray programs initiated after bulb initiation resulted in poor disease control and yield depression relative to applications beginning 2 weeks before bulb development (33). Bartolo et al. (3) simulated different levels of hail damage to onion crops at various growth stages in Colorado and found onion yield and grade to be most negatively impacted when defoliation occurred around the time of bulb initiation. Yield losses decreased with plant maturity at the time of injury. A similar phenomenon may exist for Xanthomonas leaf blight as the disease reduces the photosynthetic area of onion plants in a similar manner to mechanical damage. Late-season epidemics, as observed in 2003 and 2004, are likely to have negligible impact on bulb yield and grade, and copper hydroxide applications for management of Xanthomonas leaf blight are probably not necessary within 1 or 2 weeks of onion harvest.

In this study, effective and economical means of reducing the use of copper bactericides and EBDC fungicides for suppression of Xanthomonas leaf blight were identified. The number of copper bactericide and EBDC fungicide applications may be reduced by applying them during the critical periods for disease control, or by relying upon alternative products such as acibenzolar-*S*-methyl and biological control agents. Combining these chemical and biological control treatments into an integrated management program that emphasizes planting of pathogen-free seed (28,29), moderate nitrogen fertility (D. H. Gent and H. F. Schwartz, *unpublished data*), sanitation of weeds, leguminous crops, infested crop debris, and volunteer onion, avoids reuse of irrigation water, and follows a 2-year or longer rotation to non-host crops such as small grains, should reduce the need for class B2 carcinogens to manage Xanthomonas leaf blight in Colorado and possibly elsewhere.

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